

Effect of freezing temperature and duration on winter survival and grain yield of winter wheat

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ABSTRACT

Climate change has already increased the occurrence of extreme temperature events, which has restricted the production and development of winter wheat. To evaluate the effects of freezing temperature and duration on winter wheat survival and grain yield, two years of controlled freezing experiments were conducted under different freezing temperatures and durations. Three freezing temperatures (T1, T2 and T3) were implemented in these experiments, and soil minimum temperatures of -14°C , -17°C and -20°C were designated for T1, T2 and T3, respectively. The freezing durations were designed as 2, 4, 6, 8 and 10 days under T1, 1, 2, 3, 5, 6, 7, 8 and 9 days under T2, and 1, 2, 3, 4, 5, 6 and 8 days under T3. The effects of freezing duration on the mortality of plants and tillers, as well as its effects on the grain yield per pot (GYPP), were much more serious under lower temperature. For every 1-day increase in freezing duration, GYPP decreased by 3.3% and 8.4% under T1 and T2, respectively, while the spike number per pot (SNPP) decreased by 4.4% and 21.6% under T1 and T2, respectively. When analyzing the combined effects of freezing temperature and duration, we introduced freezing degree days (FDD), which uses soil minimum temperatures below a threshold of -12°C to represent the magnitude of the exposure to freezing temperature and duration. For every $1^{\circ}\text{C}\cdot\text{d}$ increase in FDD, the mortality of plants and tillers increased by 1.0% and 1.4%, respectively. The interaction between freezing temperature and duration had a great effect on GYPP ($0.5\text{--}1.2\%/^{\circ}\text{C}\cdot\text{d}$), while SNPP was more sensitive to freezing stress than GYPP and decreased at a rate of $2.3\text{--}2.9\%/^{\circ}\text{C}\cdot\text{d}$. An increasing trend in grain number per spike (GNPS) was clearly observed as FDD increased in 2014–2015, while freezing stress during the overwintering period had no direct influence on thousand-grain weight (TGW). The variation in GYPP was mainly caused by the grain number per pot ($\text{GNPP} = \text{SNPP} \times \text{GNPS}$). The contribution of SNPP to the variation in GYPP was greater than that of GNPS or TGW under mild temperature, while GNPS contributed more under severe temperature. The results of this study provide data support for the improvement of crop models when simulating the effect of temperature stress on grain crops, and they can be consulted to predict freezing injury during winter wheat production.

1. Introduction

Wheat is the third-largest crop in the world; it grows on more than 200 million hectares of cropland worldwide and annually produces over 600 million tons (FAO, 2013). China is currently the largest wheat-producing country in the world, as it is responsible for more than 17.6% of global wheat production (FAO, 2013). Winter wheat, which represents more than 90% of wheat produced in planting areas in China (National Bureau of Statistics of China, 2017), is exposed to climatic

vagaries for longer periods during overwintering periods, which can potentially have negative impacts on winter survival, crop vigor, and hence final yields (Holmer, 2008; Licker et al., 2013; Peltonen-Sainio et al., 2011; Reinsdorf and Koch, 2013; Vico et al., 2014). In many areas where winter wheat is grown over large areas, this crop frequently suffers freezing injury of varying severity, resulting in significant yield losses (Andrews et al., 1974; Li et al., 2005; Skinner, 2012). Therefore, it is important to evaluate the responses of winter wheat to freezing stress during overwintering periods.

Abbreviations: CDD, cooling degree days; FDD, freezing degree days; GNPP, grain number per pot; GYPP, grain yield per pot; LD₁₀, the number of freezing days required for 10% mortality; LD₂₀, the number of freezing days required for 20% mortality; LD₅₀, the number of freezing days required for 50% mortality; LT₅₀, the temperature at which 50% of plants are killed by freezing; SNPP, spike number per pot; TGW, thousand-grain weight

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The mean surface air temperature of the Earth has increased by 0.86 °C from the Industrial Revolution to recent years (Abbas et al., 2017; IPCC, 2014). Under global warming, higher winter temperatures have caused winter wheat to extend northward and westward in China and have changed the cropping system and cultivated varieties in winter wheat-planting regions (Hao et al., 2001; Li et al., 2013; Ren et al., 2005; Sun and Liu, 2008; Tan et al., 2009; Yang et al., 2011). However, climate change has already increased the occurrence of extreme climate events, especially extreme temperature (IPCC, 2014), which has influenced the stability of crop production and caused severe reductions in yield, thus restricting the development of wheat production (Lobell et al., 2011; Xiong et al., 2005; Zheng et al., 2012).

Experimental evidence has suggested that the freezing injury of winter wheat is influenced by extreme freezing temperatures (of both air and soil) and freezing durations under milder, subzero temperatures during winter and that these two factors exert different degrees of influence (Gusta et al., 1997; Roberts, 1985; Skinner and Garland-Campbell, 2008; Taylor and Olsen, 1985; Vico et al., 2014). For example, air temperature of −23 °C for one day resulted in 50% mortality of plant for Norstar, while 13 freezing days at the air temperature of −15 °C caused 50% mortality (Gusta et al., 1997). The relationships between freezing soil temperature and winter wheat mortality have been established in controlled freezing experiments, and LT₅₀ (the temperature at which 50% of plants are killed by freezing) has been determined using these relationships (Mu et al., 2015; Zhao and Liang, 1987; Zheng, 1981). In addition, mathematical models have been built to represent the relationship between freezing duration and winter wheat mortality at particular temperatures (Gong et al., 1982; Zheng, 1981).

Freezing injury also has a great influence on grain yield and yield components. The effect of freezing injury of different degrees on grain yield has also been assessed in previous studies. Different responses of grain yield and yield components were observed at varying degrees of freezing injury. When winter wheat plants were slightly injured, grain numbers per spike increased and thousand-grain weight decreased slightly. For plants whose main stem froze to death, spike number, thousand-grain weight and grain yield per plant decreased by 12.3%, 7.8% and 10.2%, respectively, whereas grain number per spike increased by 11.3%. For plants whose main stem and strong tillers both were killed by freezing, the spike number, grain number per spike, thousand-grain weight and grain yield decreased by 49.2%, 17.7%, 14.6%, and 64.3%, respectively (Zheng et al., 1989). For winter wheat fields, freezing injury during winter mainly reduced spike rate and number thus decreasing yield, but with little effect on the grain number per spike and thousand-grain weight (Barlow et al., 2015; Huangfu et al., 1996). However, previous studies have mostly investigated the effect of either freezing temperature or freezing duration alone on winter wheat survival, with less focus on the combined effects of temperature and duration. In addition, the quantitative impact of freezing temperature and duration on grain yield and yield components has rarely been reported.

Cooling degree days (CDD), a factor that combines low temperature and duration, has been employed to quantify the effects of low temperature on the growth, development and yield formation of crops (Ji et al., 2017; Ma et al., 2003; Roel et al., 2005; Shimono et al., 2005, 2011). However, these studies mostly focused on the effect of low temperatures above 0 °C on yield formation during reproductive stages. Few studies have been conducted to evaluate the combined effect of freezing temperature below 0 °C and freezing duration on winter wheat survival and grain yield during overwintering periods. Freezing degree days (FDD), was used in here to define the degree and duration of cold during the overwintering period of winter wheat. The previous study reported that the crown is considered the organ most crucial for winter survival of winter wheat, as a viable crown is required for the regeneration of other plant organs damaged by freezing injury (Persson et al., 2017). The soil temperature around the wheat crown commonly

determines whether a wheat crop will suffer a freezing injury during the winter (Persson et al., 2017). Therefore, the soil temperature was used here to calculate FDD.

In this study, two years of controlled freezing experiments were conducted using different freezing temperatures and durations during winter. The objectives were to: (1) establish the relationships between freezing duration and mortality and grain yield at different freezing temperatures, (2) quantify the combined impacts of freezing temperature and duration on winter survival and grain yield using FDD, and (3) explore the contribution of yield components to variations in grain yield at different freezing temperatures during overwintering periods.

2. Materials and methods

2.1. Experimental materials and management

The experiments were conducted during 2014–2016 at China Agricultural University, which is located in Beijing, China. Winter wheat cultivar Jing411 (strong-winter type) was used in these experiments. This wheat is widely cultivated in the northern area of the Huang-Huai-Hai region due to its strong hardiness. Winter wheat was planted in 23 cm (diameter) × 22 cm (deep) plastic pots on September 28 (2014–2015) and September 30 (2015–2016). Prior to sowing, the pots were filled with 7.5 kg of sandy loam soil that had been dried in the sun and 2.0 g (N:P:K = 15:15:15) fertilizer per pot as base fertilizer and then watered to water-holding capacity. Twenty seeds were sown at a depth of 2.5 cm in each pot and the seedlings were subsequently thinned to 10 per pot at the three-leaf stage. The pots were buried in the experimental field after sowing so that the plants could grow and acclimate under natural conditions before the freezing treatments were applied. An application of 1.0 g N per pot was conducted at the time of regrowth after the freezing treatments. Other cultivation practices, such as irrigation and pesticide application, were carried out as required, so that the experimental plants grew without stress from water, disease or pests.

The meteorological conditions during November and December in the experiment years in the experimental field, including daily air minimum temperature, daily soil minimum temperature at 2.5 cm depth and solar radiation (which influence the hardening levels of winter wheat), are shown in Fig. 1. The average air minimum temperature was 1.8 °C in November and −3.7 °C in December in the year 2014, while the average air minimum temperature was 1.3 °C in November and −2.4 °C in December in 2015. The average soil minimum temperature was 2.0 °C in November and −3.8 °C in December in 2014, while the average soil minimum temperature was 2.6 °C in November and −1.8 °C in December in 2015. The controlled treatments were conducted during late December to next January. The air and soil minimum temperatures were −3 °C to −6 °C respectively, in the experimental field before the treatments.

2.2. Controlled freezing treatments and observations

The experimental process is shown in Fig. 2. In order to expose the winter wheat cultivar to freezing injury of different degrees, three freezing temperature regimes (T1, T2 and T3) representing different freezing temperature levels were implemented and soil minimum temperatures ($T_{\min}$) for T1, T2 and T3 were −14 °C, −17 °C and −20 °C, respectively. The mortality of plants was 0%, 5% and 30% under T1, T2 and T3 for one day, respectively (Mu et al., 2015). Once the targeted minimum temperature was established, the diurnal temperature range and the rate of cooling were adjusted. The respective soil maximum temperatures ($T_{\max}$) for T1, T2 and T3 were established at −5 °C, −8 °C and −11 °C, in order to ensure that there were no death of plants and tillers due to the soil maximum temperatures and the diurnal temperature range. Experimental plants that had acclimated outside were moved to the freezing chamber for controlled freezing

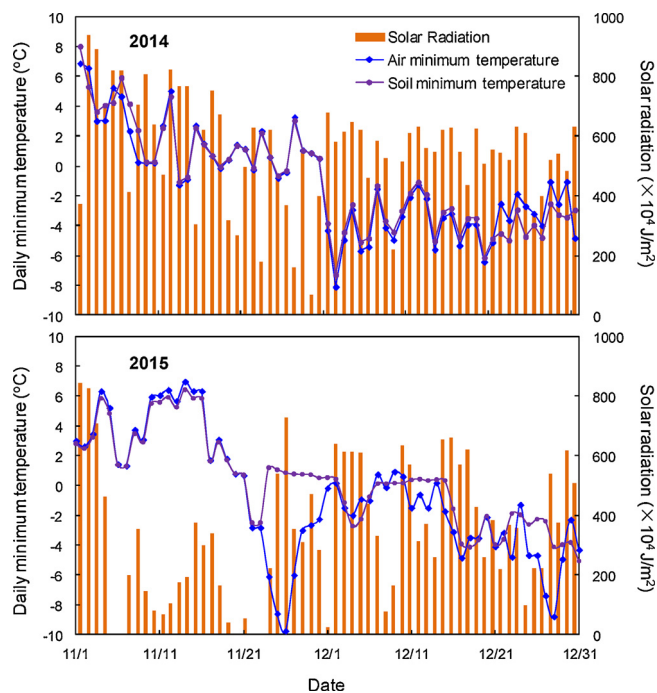


Fig. 1. Daily air minimum temperature, daily soil minimum temperature at 2.5 cm depth, and solar radiation in the experimental field during November and December of 2014 and 2015.

treatments during winter, i.e. during dormancy of winter wheat (Zadoks 26). The pots were placed in the freezing chamber at random and rotated every day to minimize positional effects. To simulate daily changing patterns in soil temperature in the field when freezing injury occurred as closely as possible, the temperature in the freezing chamber decreased at a cooling rate of approximately 1.0°C/h , which was consistent with the soil temperature in the pots and a little faster than the cooling rate of soil temperature in the field in average years in order to reach the targeted soil minimum temperature ($T_{\min}$) and was held there for 3 h. The temperature was then raised at a similar rate to the soil maximum temperature ($T_{\max}$) and maintained there for 3 h, followed by repeated cooling and warming cycles for several days. During the winter of 2014–2015, the freezing durations were set as 2, 4, 6, 8 and 10 days under T1; 5, 6, 7, 8 and 9 days under T2; and 2, 4, 5, 6 and 8 days under T3, in order to expose the winter wheat samples to different cumulative freezing degrees under each freezing temperature level. During the winter of 2015–2016, the freezing durations under T1 were the same as that in 2014–2015, while the freezing durations were

revised to 1, 2, 3 and 5 days under T2; and 1, 2, 3, 4 and 5 days under T3, so as to improve the experimental effectiveness and obtain LD_{10} , LD_{20} and LD_{50} (the number of freezing days required for 10%, 20% and 50% mortality) values. When the winter wheat was applied treatment in the freezing chamber, the soil temperature in the pots was monitored at a depth of 2.5 cm using temperature probes AV-10 T (Avalon Scientific, Inc. USA).

After treatment, the experimental plants were allowed to thaw under standard conditions of approximately 4°C for 2d at a 10-h photoperiod that resembled outdoor light conditions. They were then transferred to a greenhouse at a temperature of approximately 25°C for regrowth and the analysis of yield formation. A control group for each cultivar in each year, which did not experience a prolonged freezing treatment, was held at approximately 4°C for 2d at a 10-h photoperiod and maintained in the greenhouse until harvest. There were three replicates for each treatment and the control group.

The numbers of plants and tillers per pot prior to the freezing treatment and those surviving after freezing treatment were recorded as A1 and A2, respectively. The mortality of plants and tillers in each pot was calculated as $y = (A1 - A2) / A1$. The grain yield and yield components were measured at maturity in the greenhouse, including grain yield per pot (GYPP), spike number per pot (SNPP), grain number per spike (GNPS), and thousand-grain weight (TGW).

2.3. Statistical analysis

The following equation was used to establish the relationships between the mortality of winter wheat plants and tillers and freezing duration at the various freezing temperatures (Zheng, 1981):

$$y = 1 - (1 - P)^{(n - K)} \quad (1)$$

In Eq. (1), y (%) is the mortality of plants and tillers and n (d) is the freezing duration. The equation was determined through controlled freezing experiments, and the simulated result from the equation was validated with the measured data (Zheng, 1981). Furthermore, both parameters in the equation are biological. The parameter K (d) is the maximum number of ineffective freezing days, which represents the number of days that plants and tillers can stay alive under different temperatures. The parameter P (%/d) is the death rate, which reflects the increasing mortality of living plants and tillers for each additional effective freezing day. However, with climate change and cultivars renewal, the parameters of current cultivars were different from those of older cultivars. The parameters K and P were determined for the current cultivar J411 by our experiment. Simple linear regressions were used to evaluate the effects of freezing duration on grain yield and yield components at different freezing temperatures. In order to avoid abnormal values misleading the results, the treatments under which mortality of

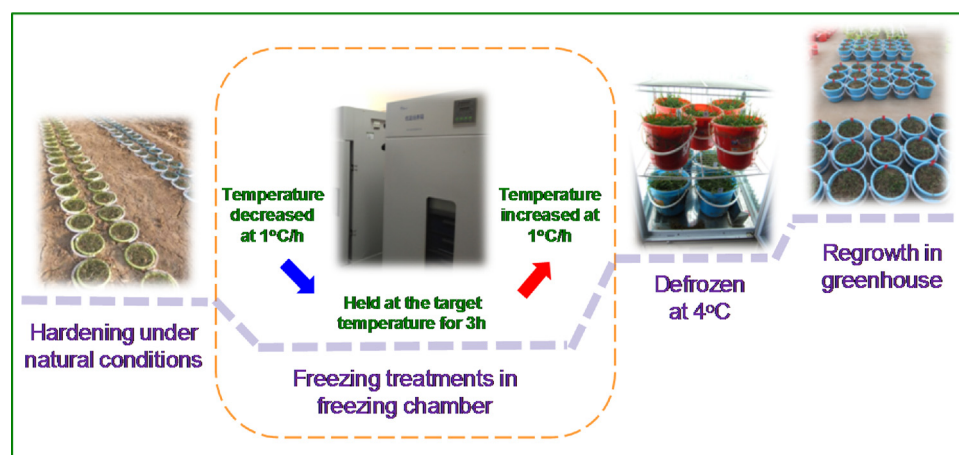


Fig. 2. Schematic diagram of experimental process.

plants and tillers was more than 5% and lower than 95% were included in the result analysis (Fowler, 1979). Thus, there were two effective treatments of freezing durations under T3 in each year, so the relationship between freezing duration and mortality of plants and tillers was established under T1 and T2, as well as the linear regression analysis on the effects of freezing duration on grain yield and yield components.

During the analysis of the combined effects of freezing temperature and duration, freezing degree days (FDD) were used to record the magnitude of exposure to freezing stress during the overwintering periods in this study. It was calculated as:

$$FDD = \sum_{i=1}^n (T_h - T_i) \quad (2)$$

T_h (°C) is the freezing temperature threshold set at -12°C (Yu, 1982). T_i (°C) is the soil minimum temperature at the depth of 2.5 cm, in the pots on the i th day of the freezing treatments.

Simple linear regressions were used to quantify the combined impacts of freezing temperature and duration on winter survival and grain yield using FDD. Then, FDD values required for 10%, 20% and 50% mortality of plants and tillers were obtained from the fitted linear regressions. The relative values of GYPP, SNPP, GNPS and TGW were used to standardize the responses to freezing temperature and duration, and were calculated as the ratios of the absolute values obtained from the different treatments to the corresponding values obtained from the controls (i.e., the samples without exposure to the freezing treatment). Furthermore, multiple linear regressions were used to quantify the contribution of yield components to the variations in grain yield.

3. Results

3.1. Effect of freezing duration on winter survival and grain yield at different freezing temperatures

3.1.1. Effect of freezing duration on winter survival

Freezing duration had a substantial effect on winter wheat survival, and its effect differed with freezing temperatures. Photographs of some samples of the winter wheat after T1 and T2 freezing treatments are presented in Fig. 3. Freezing injury increased with longer freezing durations at each freezing temperature, and also for the same duration at increasingly lower temperature. For example, the freezing injury of winter wheat was more serious after 2 days under T2 than that under T1. And the freezing injury of winter wheat after 3 days under T2 was more serious than it was after 6 days under T1.

The relationships between freezing duration and mortality of plants

and tillers under T1 and T2 are shown in Fig. 4. The mortality of plants and tillers was significantly associated with freezing duration, and it increased at different rates as freezing duration increased. Mortality and death rate were greater under lower temperature for plants and tillers (Fig. 4 and Table 1). For plants, death rate was 9%/d under T2, while there was no death under T1 for 2–10 days. For tillers, death rate was 9%/d greater under T2 than under T1. LD_{50} values (the number of freezing days required for 50% mortality) were obtained from the relationship curves in Fig. 4 and are recorded in Table 1. The results reveal that LD_{50} was 6.3 days longer for plants than that for tillers under T2. In contrast to the response of plants, there was mortality of tillers in T1 but with LD_{50} 9.2 days longer than under T2.

3.1.2. Effect of freezing duration on grain yield and yield components

Simple linear models were used to analyze the effects of freezing duration on grain yield and its components at different freezing temperatures (Table 2). These results reveal that GYPP decreased as freezing duration increased. The freezing temperature had a great impact on the effect of freezing duration on grain yield and its components. The effects of freezing duration on GYPP were much more serious under lower freezing temperature. For every 1-day increase in freezing duration, GYPP decreased by 3.3% and 8.4% under T1 and T2, respectively. SNPP exhibited a more rapidly decreasing response with rates of 4.4%/d and 21.6%/d under T1 and T2, respectively. With increasing freezing duration, responses of GNPS differed between freezing temperatures. An increasing trend of GNPS was observed under T2, while decreasing trends were observed under T1. The freezing duration exerted no significant effect on TGW at all freezing temperatures.

3.2. Quantifying the combined effects of freezing temperature and duration on winter survival and grain yield

3.2.1. Relationships between FDD and mortality of plants and tillers

Significant relationships were observed between freezing degree days (FDD) and the mortality of plants and tillers (Fig. 5A). The interaction of freezing temperature and duration had a slightly greater effect on survival of tillers than on plants. The mortality of tillers was greater than that of plants at the same FDD. For every $1^{\circ}\text{C}\cdot\text{d}$ increase in FDD, mortality increased by 1.0% and 1.4% for plants and tillers, respectively. It was observed that 10%, 20% and 50% of plants died when the FDD was $6.0^{\circ}\text{C}\cdot\text{d}$, $16.0^{\circ}\text{C}\cdot\text{d}$ and $46.0^{\circ}\text{C}\cdot\text{d}$, respectively, while 50% of tillers died when the FDD was $18.6^{\circ}\text{C}\cdot\text{d}$ (Fig. 5B).

3.2.2. Relationships between FDD and grain yield and yield components

To evaluate the combined effects of freezing temperature and

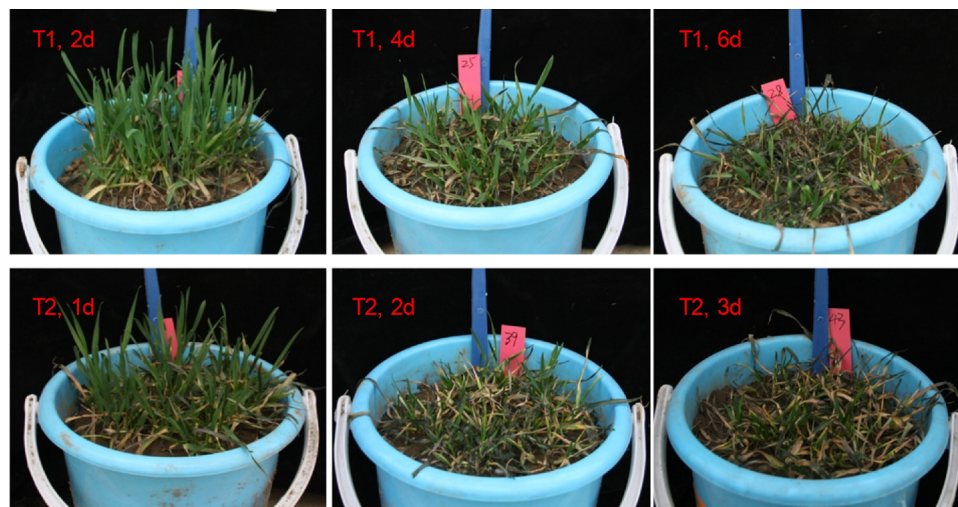


Fig. 3. Photographs of winter wheat after undergoing freezing treatments under different freezing temperatures and durations.

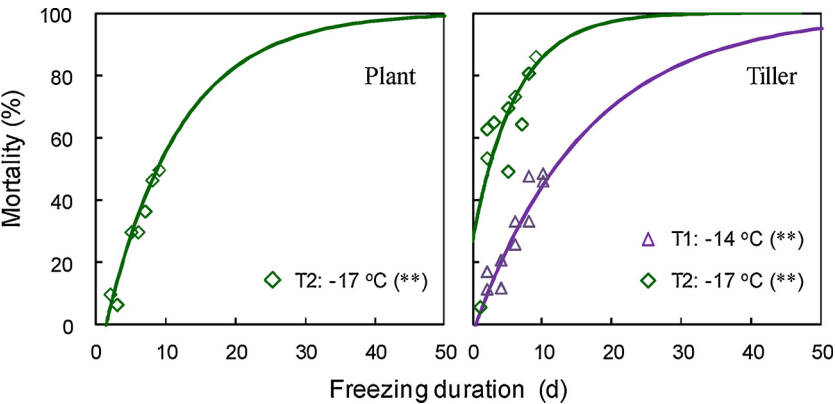


Fig. 4. Relationships between freezing duration and mortality of plants and tillers at different freezing temperatures. Symbols indicate the values measured in the controlled freezing treatments, and solid lines indicate the relation curves between freezing duration and the mortality of plants and tillers. ** the regression curves was significant at $P < 0.01$.

Table 1
Death rates and LD₅₀ values for plants and tillers at different freezing temperatures.

Freezing temperature	Death rate (%/d)		LD ₅₀ (d)	
	Plant	Tiller	Plant	Tiller
T1	/	6	/	11.6
T2	9	15	8.7	2.4

LD₅₀: the number of freezing days required for 50% mortality of plants and tillers.

Table 2
Linear regression analysis between relative GYPP, SNPP, GNPS, TGW and freezing duration at different freezing temperatures.

Freezing temperature	Year	Relative GYPP	Relative SNPP	Relative GNPS	Relative TGW
T1	2014–2015	–3.3	–4.2	–1.7	0.6
	2015–2016	–3.3	–4.6	–0.4	–2.3
T2	2014–2015	–10.9**	–21.4**	10.4**	–2.5
	2015–2016	–5.9*	–21.8	6.2	0.9

GYPP-grain yield per pot; SNPP-spike number per pot; GNPS-grain number per spike; TGW-thousand-grain weight.

* The linear regression coefficient was significant at $P < 0.05$.
** The linear regression coefficient was significant at $P < 0.01$.

duration on yield formation, relationships were established between freezing degree days (FDD) and grain yield and yield components, including grain yield per pot (GYPP), spike number per pot (SNPP), grain number per spike (GNPS) and thousand-grain weight (TGW), as shown in Fig. 6. Compared with the controlled group, SNPP was greater when FDD was less than 28.5 °C·d in 2014–2015 and 14.7 °C·d in 2015–2016, while GNPS was greater when FDD was greater than 17.1 °C·d in

2014–2015. In contrast, GYPP and TGW were both inhibited by the interaction of freezing temperature and duration.

As is shown in Fig. 6, the trends exhibited by GYPP, SNPP, GNPS and TGW differed as FDD increased. GYPP decreased significantly as FDD increased, but differences were observed between two years. At the same FDD, the GYPP in 2014–2015 was greater than that in 2015–2016. The decreasing trend of GYPP resulted from the decrease in SNPP. The interaction of freezing temperature and duration had a greater effect on SNPP than it did on GYPP. However, the difference in SNPP between two years was smaller than that of GYPP. For every 1 °C·d increase in FDD, SNPP decreased by 2.3% and 2.9% in 2014–2015 and 2015–2016, respectively. In 2014–2015, with increasing FDD, GNPS significantly increased at a rate of 1.6%/°C·d, while GNPS exhibited no obvious increasing trend in 2015–2016. There was no obvious trend in TGW with increasing FDD.

3.3. Relationship between grain yield and yield components at different freezing temperatures

Multiple linear regression analysis was used to evaluate the relationship between grain yield (GYPP) and yield components (SNPP, GNPS and TGW). The results are presented in Table 3. Grain number per pot (GNPP = SNPP × GNPS) contributed more to GYPP than did TGW. Under T1, T2 and T3, GNPP contributed 86.7%, 93.6% and 77.4% of the total variation in GYPP, respectively. The contribution of TGW to GYPP was less than 20% under T1 and T2. In addition, the relationship between GYPP and each yield component was evaluated at all freezing temperatures to compare the contributions of SNPP and GNPS to GYPP. The contributions of SNPP and GNPS to GYPP differed at different freezing temperatures. In comparison, the contribution of SNPP to GYPP was greater than that of GNPS and TGW under T1, while the contribution of GNPS was slightly greater than that of SNPP under T2 and substantially greater than that of SNPP under T3. The variability in SNPP explained most of the observed variability in GYPP under T1

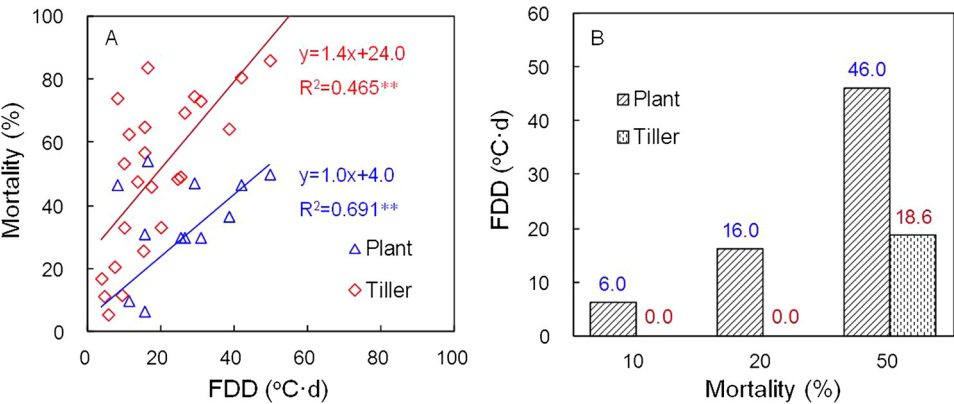


Fig. 5. (A) Linear relationships between mortality of plants and tillers and freezing degree days (FDD) and (B) FDD values for three levels (10%, 20% and 50%) of mortality of plants and tillers. Symbols indicate the values measured in controlled freezing treatments, and solid lines indicate the relation curves between FDD and the mortality of plants and tillers. ** $P < 0.01$.

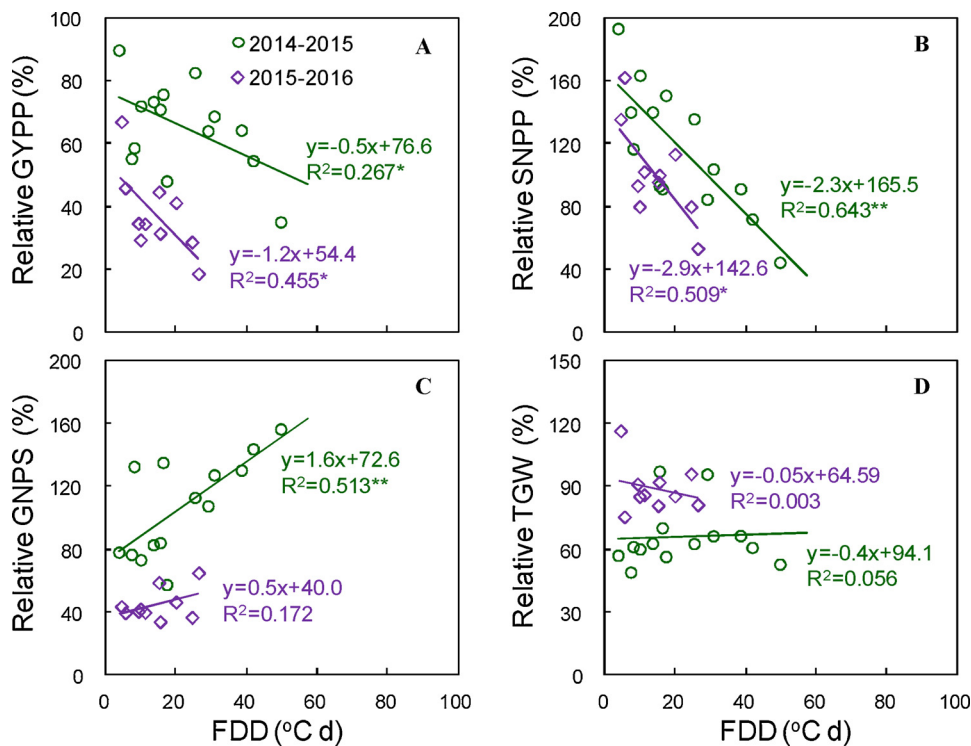


Fig. 6. Relationships between relative grain yield per pot (GYPP) (A), spike number per pot (SNPP) (B), grain number per spike (GNPS) (C) and thousand-grain weight (TGW) (D) and freezing degree days (FDD). Symbols indicate the relative GYPP, SNPP, GNPS and TGW measured in the controlled freezing treatments, and solid lines indicate the relation curves. * $P < 0.05$, ** $P < 0.01$.

Table 3

Multiple linear regression analysis between grain yield and yield components.

Yield components	T1 (n = 10)		T2 (n = 10)		T3 (n = 4)	
	SPRC	Partial R ²	SPRC	Partial R ²	SPRC	Partial R ²
SNPP	0.893 ^{**}	0.766	0.826 ^{**}	0.326	2.109	0.131
GNPS	0.411 ^{**}	0.088	0.712 ^{**}	0.518	5.422	0.672
TGW	0.384 ^{**}	0.128	0.365 ^{**}	0.125	4.537	0.196
GNPP	1.046 ^{**}	0.867	0.878 ^{**}	0.936	1.581 [*]	0.774
TGW	0.376 ^{**}	0.128	0.257 ^{**}	0.058	0.846	0.224

SNPP-spike number per pot; GNPS-grain number per spike; TGW-thousand-grain weight; GNPP-grain number per pot; SPRC-standardized regression coefficient.

* The coefficient was significant at $P < 0.05$.

** The coefficient was significant at $P < 0.01$.

(76.6%), while it explained only 32.6% and 13.1% of the observed variability in GYPP under T2 and T3, respectively. GNPS contributed to approximately 8.8%, 51.8% and 67.2% of the variations in GYPP under T1, T2 and T3, respectively. It can thus be estimated that in these experiments, the contribution of GNPS to variations in GYPP was greater under lower freezing temperatures.

4. Discussion

Although the effects of freezing temperature and freezing duration at particular temperatures have been evaluated on winter wheat survival by many studies, few have focused on the combined effects. In the present study, the parameter freezing degree days (FDD) was established to evaluate the comprehensive impacts of freezing temperature and duration on winter wheat survival and grain yield. In previous studies, cooling degree days (CDD) was widely used to quantify the effects of low temperature and duration on rice yield. For this, CDD was calculated as the number of days when daily mean temperature fell below a temperature threshold (Shimono et al., 2005, 2007; Shimono, 2011). However, because winter wheat survival is mainly influenced by soil minimum temperature during the overwintering period (Persson

et al., 2017), soil minimum temperature was used to calculate FDD. This work extends the previous studies by quantifying the effects of freezing duration on winter wheat at different freezing temperatures and the combined effects of freezing temperature and duration on winter wheat survival and grain yield. Consequently, the results presented here can be applied to explain the effect of freezing temperature events on winter wheat in different years and in regions of various temperature conditions where freezing injury occurred during overwintering periods.

In this study, SNPP was greater than that observed in the controlled group when FDD was less than 28.5 °C·d in 2014–2015 and 14.7 °C·d in 2015–2016 (Fig. 6B). When the strong tillers were injured by the relatively mild freezing stress but did not die, they lost their advantage in forming spikes, and more young tillers may develop into spikes. However, young tillers grew within a relatively short period, which would have influenced biomass accumulation and grain filling and may have thus resulted in GNPS and TGW smaller than those observed in the controlled group (Fig. 6C and 6D). GNPS was observed greater than that in the controlled group when FDD was greater than 17.1 °C·d in 2014–2015 to compensate for the lack of SNPP (Slafer et al., 2014). TGW was mainly determined by the grain filling stage after anthesis (Ji et al., 2017); thus, freezing stress had no direct effect on TGW during overwintering periods. However, because the yield components are developmentally correlated, TGW was indirectly influenced by the grain number per pot ($GNPP = SNPP \times GNPS$) (Sadras and Denison, 2009; Slafer et al., 2014). Whatever, GYPP under freezing treatment was less than that observed in the controlled group, and decreased with increasing FDD. In this study, when the FDD was 16.0 °C·d, the mortality of plants was 20%, and the relative GYPP was 67% in 2014–2015, indicating that 20% mortality of plants resulted in 33% reduction in grain yield. When the FDD was 46.0 °C·d, the mortality of plants was 50%, and the relative GYPP was 52% in 2014–2015, indicating that 50% mortality of plants resulted in 48% reduction in grain yield. This was consistent with the previous study (Zhu, 1986) in which the experiment was conducted in field, and 20% and 50% mortality of plants caused 26% and 44% reduction in grain yield respectively.

The standardized regression coefficient has been widely used to

evaluate the contribution of yield components to variations in grain yield (Anzoua et al., 2010; Ji et al., 2017; Talukder et al., 2014). The results of this study demonstrated that GNPP (SNPP $\times$ GNPS) contributed more than TGW at all freezing temperatures, which is consistent with the results observed in the jointing and booting stages by Ji et al. (2017). Nonetheless, the contributions of SNPP and GNPS to GYPP differed between freezing temperature treatments. The contribution of SNPP to GYPP was greater than that of GNPS under mild freezing (T1), while GNPS contributed more to GYPP than SNPP under severe freezing (T2 and T3), which was inconsistent with the results obtained at the jointing and booting stages (Ji et al., 2017). This might be due to differences in the impacts of temperature stress on individual yield components during the different stages. Under T1, decrease in GYPP was mainly induced by SNPP, which was associated with tiller death. Under T2, the variation in GYPP was complexly influenced by the decrease in SNPP and the increase in GNPS. Under the most severe temperature stress (T3), the variation in GYPP was mainly influenced by substantial decreases in both SNPP and GNPS. Differences in winter survival were detected as differences in the death rate at the various freezing temperatures. The death rates of both plants and tillers were greater under severe temperatures, as is visually evident in Fig. 3. This shows that severe temperature aggravated the negative impacts of freezing duration.

In this study, the combined effects of freezing temperature and duration on grain yield and yield components differed between the two experimental years. This may have been caused by differences in the hardening periods prior to treatment, as temperature and solar radiation in late autumn determine the conditions for hardening. As is shown in Fig. 1, the acclimation conditions in 2015–2016, with little solar irradiance and relatively high temperatures in middle November, provided less hardening than in 2014–2015. More seriously, the sudden drop in daily minimum temperature in late November of 2015–2016 may have injured, if not even killed, some plants, which intensified the influence of meteorological conditions before freezing treatments on the controlled experiment. Both effects could increase the negative impacts of subsequent controlled freezing treatments on grain yield and yield components. Therefore, the values of GYPP, SNPP and GNPS shown in Fig. 6 were relatively lower in 2015–2016 than in 2014–2015, and the decreasing rates of GYPP and SNPP were greater in 2015–2016 than 2014–2015.

In recent years, many crop simulation models that simulate the growth and yield development of winter wheat in response to environmental factors have been published (Martre et al., 2015; Rickman et al., 1996; Semenov and Porter, 1995). However, few of the models include effects of freezing injury and winter survival thus limiting their applicability to years when freezing injury occurred (Bergjord Olsen et al., 2018). Inclusion of the relationships established here of the effects of freezing temperature and duration on winter wheat survival and grain yield provide an opportunity to improve the range of applicability of these crop simulation models to colder environments. The results of this study can also be applied to the prediction of freezing injury and the development of adaptation strategies during winter wheat production under climate change. If as proposed, the different results obtained in two years reflect the influence of acclimation conditions on levels of freezing stress, then adaptation strategies should vary according to seedling quality and hardening levels. The effects of freezing stress on winter wheat in fields are influenced by a variety of factors, including cultivar, field management, soil conditions and climatic conditions (Reynolds et al., 2011). And it may be difficult to distinguish the separate effects of each factor, as different combinations of unfavorable conditions may occur together in one winter (Hayhoe et al., 2003). Controlled freezing experiments allow replication over time and can evaluate the separate effects of freezing temperatures and durations on winter survival. This experiment was conducted under controlled conditions without water, disease and pest stresses, and can reflect response of winter wheat to freezing stress under such conditions.

However, the variety of factors that influence winter survival in the field can't be reflected in one experiment. For example, the factors such as cyclic freezing and thawing, snow mold, soil heaving, and ice encasement, which can occur together in one winter and cause the responses of winter wheat to freezing stress to differ from those observed under the controlled conditions, were not included in this controlled freezing experiment. Furthermore, climate change can also increase the difficulty in accurately predicting future freezing temperature events and their influences (Bergjord Olsen et al., 2018). Therefore, it is necessary to systematically study and more comprehensively understand plant phenology, physiology, survival, yield formation and their responses to climate factors.

5. Conclusions

The effects of freezing temperature and duration on winter wheat survival and grain yield were evaluated and the factor freezing degree days (FDD) was introduced to quantify their combined effects. The results reveal that the effects of freezing duration on the mortality of plants and tillers, as well as its effects on the grain yield per pot, increased greatly with decreasing temperature. For every 1-day increase in freezing duration, GYPP decreased by 3.3% and 8.4% under T1 (-14°C) and T2 (-17°C), respectively, while SNPP decreased by 4.4% and 21.6% for the same sequence. The interaction of freezing temperature and duration had a slightly greater effect on the mortality of tillers ($1.4\%/^{\circ}\text{C}\cdot\text{d}$) than of plants ($1.0\%/^{\circ}\text{C}\cdot\text{d}$). The combination of freezing temperature and duration had a large effect on GYPP ($0.5\text{--}1.2\%/^{\circ}\text{C}\cdot\text{d}$) but more so on SNPP ($2.3\text{--}2.9\%/^{\circ}\text{C}\cdot\text{d}$). However, the increasing trend in GNPS can compensate for yield loss to some extent, as FDD increased at temperature that was not extremely severe (under T2 treatment). Freezing stress during the overwintering periods had no direct influence on TGW. GNPP contributed more to the variation in GYPP (77.4–93.6%) than TGW at all freezing temperatures. SNPP contributed most (76.6%) to the variation in GYPP under the mild temperature (T1), while the contribution of GNPS increased and exceeded that of SNPP as the freezing temperature became more severe. The results of this study can be consulted to improve the accuracy of yield simulation using crop models in the regions where freezing injury occur occasionally in the future. However, winter survival in the field was influenced by a variety of factors, so it is necessary to systematically study and more comprehensively understand tolerance of winter wheat to freezing stress under various conditions.

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